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Review article

Supplementation of ram semen extender to improve seminal quality and fertility rate

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ABSTRACT

In sheep, artificial insemination serves as an important technique for breed improvement. In this context, genetic material from a small number of superior sires can be used in a large number of females. During this process, the storage of ram sperm may influence the efficiency of artificial insemination. Two main methods are currently used for ram semen storage: liquid storage and cryopreservation. The oxidative stress during the storage process can injure ram sperm and in some cases this leads to irreversible damage at the cellular level. To reduce such negative effects, different preservation protocols, extenders and protective components have been tested to improve ram sperm quality and to achieve greater fertility rates. This review provides an overview of the recent progress in extender supplementation using antioxidants and other compounds to improve ram semen quality parameters and fertility rates. It will emphasize on enzymes, vitamins, amino acids, proteins, some plant extracts and other compounds such as sugars, seminal plasma and fatty acids that can be used to supplement the extenders to reduce the formation of oxidants in ram semen and maintain its quality and enhance its fertility. It will also stress on how these supplements act, what were the tested levels giving beneficial effects on motility, viability, plasma membrane integrity and DNA fragmentation in liquid, cooled and post-thawing semen?

1. Introduction

The process of artificial insemination (AI) in sheep (*Ovis aries*) occurs as a result of a combination of several actions. One of the most important steps in this process is semen preservation in both liquid and frozen forms. Handling semen generally causes a gradual decrease in both quality (sperm motility, viability and functional integrity of ram sperm membranes; Maxwell and Salamon, 1993; Salamon and Maxwell, 1995, 2000; De Lamirande et al., 1997; Gillan et al., 1997; Azevedo, 2006; Maia, 2006, 2009; Rodello, 2006; Mahfouz et al., 2010) and fertility (Maxwell and Salamon, 1993; Vishwanath and Shannon, 1997). Sperm are extremely susceptible to low temperatures during the cooling or freezing process. This has been attributed to the high concentrations of polyunsaturated fatty acids in the plasmatic membrane of ram sperm (Jones and Mann, 1976; White, 1993; Buhr et al., 1994) that renders the cells sensitive to cold shock as well as to lipid peroxidation in the presence of reactive oxygen species (ROS; Alvarez and Storey, 1983; Alvarez et al., 1987; Griveau et al., 1995; Holt, 2000; Watson, 2000; Samadian et al., 2010). Sperm generate ROS as a normal consequence of oxidative metabolism and a low concentration of ROS has an important role in mammalian sperm functions, like, capacitation, the

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acrosome reaction, and stabilization of the mitochondrial capsule for the midpiece (Alvarez and Storey, 1984; Aitken and Fisher, 1994; Kodama et al., 1996; Griveau and Le Lannou et al., 1997; Baumber et al., 2000). To maintain proper physiological activities a fine balance between ROS production and recycling around sperm cells is essential. Any imbalance can impair sperm function through oxidative stress, leading to increased rates of lipid peroxidation and consequently to loss of motility during prolonged storage (Aitken, 1995; Gibb and Aitken, 2016). During processing, ram sperm produce large amounts of hydrogen peroxide (Maia et al., 2010; La Falci et al., 2011), which decreases post-thawing sperm motility (Maia et al., 2014).

Successful sperm storage (liquid and frozen) requires slowing of the cell metabolism and thereby prolongs viability (Maxwell and Salamon, 1993; Yoshida, 2000; Gibb and Aitken, 2016). To achieve this goal and to improve sperm quality, the use of a suitable extender and cryoprotectant and an appropriate cooling/warming process (Fiser, 1991) are necessity. For this purpose, various components have been added to extenders to maintain motility and fertilization capacity and to preserve the integrity of the sperm membrane (Sarlos et al., 2002; Riha et al., 2006; Bucak and Tekin, 2007; Maia et al., 2009; Coyan et al., 2010; AminiPour et al., 2013). In most cases, these protectants have antioxidant activity and either reduce the process of oxidation (Pietta, 2000), or regulate, suppress or prevent the formation of ROS (Sikka, 2004; Maneesh and Jayalekshmi, 2006). Supplementation with antioxidants and other compounds, therefore, may reduce the negative effect of oxidative stress caused by ROS on ram sperm during the preservation process (Maxwell and Stojanov, 1996; Upreti et al., 1997; Bucak et al., 2007; Coyan et al., 2010; Forouzanfar et al., 2010). This review provides an overview of the recent progress in extender supplementation using antioxidants and other compounds to improve ram semen quality parameters and fertility rates.

2. Antioxidants

Mammalian sperm have an antioxidant defense system that includes superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione reductase (GR), as well as non-enzymatic antioxidants such as methionine, ascorbic acid and α-tocopherol (Mann and Lutwak-Mann, 1981; Kantola et al., 1988; Aitken, 1995; Mara et al., 2005; Bucak et al., 2012). The biosynthetic capacity of sperm, however, is limited (Aitken, 1995), and the concentration of the antioxidants present in the semen can be reduced by dilution, and as a result decreasing the beneficial effect of this endogenous antioxidative defense. Thus, the addition of antioxidants, even in small concentrations, can improve sperm function during preservation.

2.1. Enzymes

Enzymatic antioxidants are macromolecules that protect cells against ROS (Barreiros et al., 2006). The enzyme superoxide dismutase (SOD) in the cytoplasm (Cu, Zn - SOD) and in the mitochondria (Mn-SOD) is responsible for combining two molecules of the superoxide anion (O_2^-) into one of hydrogen peroxide (H_2O_2), and catalase (CAT) and glutathione peroxidase (GSH-Px) converts of H_2O_2 to H_2O and O_2 (Nordberg and Arnér, 2001; Amidi et al., 2016). This enzyme has been detected in ram semen (Marti et al., 2003; Kasimanickam et al., 2006; Bucak et al., 2008; Marti et al., 2008). Its antioxidant capacity, however, changes with semen quality (Kasimanickam et al., 2006) as well as during the freezing-thawing process (Bucak et al., 2008; Marti et al., 2008). Such changes in enzyme activities in semen as well as the relationship (positive or negative) with sperm quality can be due to oxidative stress, because enzymes are utilized in excess to protect or maintain sperm quality, or because the enzymes do not have the capacity to maintain sperm quality (Bilodeau et al., 2001; Kasimanickam et al., 2006).

In fresh semen, the SOD is the main active enzyme while the glutathione reductase (GSR) and GSH-PX are low (Kasimanickam et al., 2006; Marti et al., 2008). Furthermore, Kasimanickam et al. (2006) reported that GPx activity in ram sperm was greater in ejaculates with poor sperm quality while SOD activity did not vary with seminal quality. After incubation at 15 °C for 6 h, there was no reduction in the activity of SOD and GSH-Px (Marti et al., 2003). After a freeze-thawing cycle, however, the activity of GSR and GSH-Px remained stable, while SOD activity was about half that observed in fresh and refrigerated semen (Marti et al., 2008). In frozen ram semen, Bucak et al. (2008) detected lesser activity of GSH-Px and CAT.

Controversial results have been reported regarding the inclusion of SOD in extenders. Maxwell and Stojanov (1996) and Forouzanfar et al. (2013) reported that addition of 800 U/mL or 150 μM of SOD to the extenders provided greater protection to ram sperm cells submitted to refrigeration. However, Silva et al. (2011) did not found any enhancement in sperm kinematic parameters when adding SOD (25, 50 and 100 U/mL) to the freezing extender, but ultrastructural analysis revealed that addition of 100 U/mL of SOD preserved the integrity of the acrosome, and enhanced preservation of the mitochondria.

Catalase (CAT) has been used in extenders to improve the antioxidant capacity of semen and preserve sperm function (Maxwell and Stojavone, 1996; Upreti et al., 1998; Maia et al., 2009). Maia (2006) observed that there was a greater percentage of intact plasma and acrosome membranes when ram semen was cryopreserved with a Tris-hydroxymethyl aminomethane (Tris) egg yolk extender containing 50 μg/mL of catalase. Moreover, the inclusion of 100 and 200 U/mL of catalase in diluents can prevent the harmful effects of cooling on total motility (Câmara et al., 2011a) and on survival of ram sperm (Maxwell and Stojanov, 1996) during liquid storage at 5 °C. Nevertheless, concentrations of catalase greater than 200 U/mL were toxic to the sperm.

Most enzymatic systems for the control of cellular peroxide concentrations consist of glutathione peroxidases and several ancillary enzymes required for the synthesis and reduction of glutathione (GSH). Amid et al. (2016) reported in a review that glutathione peroxidase (GSH-Px, GPx) acts upon GSH to reduce hydrogen peroxide to H_2O and lipoperoxides to alkyl alcohols. The GSH subsequently can be regenerated from its oxidized form (GSSG) by glutathione reductase (GSR), the activity of which is inducible upon oxidative stress.

In fresh semen, the addition of GSH also had no effect on the kinematic parameters of ram sperm (Câmara et al., 2011b).